REVIEW

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Metabolism of stromal and immune cells in health and disease

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Cancer cells have been at the centre of cell metabolism research, but the metabolism of stromal and immune cells has received less attention. Nonetheless, these cells influence the progression of malignant, inflammatory and metabolic disorders. Here we discuss the metabolic adaptations of stromal and immune cells in health and disease, and highlight how metabolism determines their differentiation and function.

Stromal cells are connective cells that support parenchymal cells. Aside from endothelial cells (ECs), which build blood vessels to supply oxygen and nutrients[1,2], other stromal cells include fibroblasts, which maintain the structural framework in tissues. Another class of cells that maintains tissue homeostasis is immune cells. The immune system collectively functions to protect tissues from infections and foreign antigens. Notably, several of these cell types not only contribute to disease progression but also to therapy resistance[3].

Cancer cells undergo numerous metabolic adaptations; a result of oncogenic transformation, gene mutations or altered expression of metabolic enzymes[4–7]. Metabolism of stromal and immune cells has received less attention, yet they represent an abundant cell population in tissues and tumours, even up to 80–90% in some desmoplastic tumours[8]. We focus on two types of immune cells (T cells and macrophages) and two types of stromal cells (ECs and fibroblasts), for which metabolism has been best characterized. Emerging evidence indicates that metabolism not only co-determines their differentiation and function, but also that metabolic changes in these cells contribute to the pathogenesis of cancer, diabetes and inflammatory diseases.

Vascular endothelial cells

ECs are quiescent for years, but when tissues are deprived of oxygen or nutrients, they sprout to vascularize tissues. Vessel sprouting is a highly coordinated process, relying on a migrating endothelial 'tip' cell at the forefront that guides the vessel sprout (but rarely proliferates), and on trailing endothelial 'stalk' cells elongating the sprout via proliferation[1,9]. Vascular endothelial growth factor (VEGF) is a tip cell signal, whereas Delta-like 4 (DLL4)/Notch signalling is a stalk cell signal. Tip versus stalk cell phenotypes are not predetermined cell fates, but are interchangeable. By competing for the tip position, ECs continuously secure optimal fitness to lead the sprout. Although many genetic signals regulate this process, the role of EC metabolism in vessel sprouting has only recently been considered. We postulated that this angiogenic switch requires a metabolic switch[10].

Endothelial cells are glycolytic

ECs have immediate access to oxygen in the blood, but do not take advantage of its availability to adopt an oxidative metabolism. Instead, oxygen consumption is lower and the mitochondrial volume fraction is smaller than in oxidative cell types[11]. Also, inhibition of mitochondrial ATP production or stimulation of oxygen consumption does not affect vessel sprouting[10]. This is in line with reports that EC mitochondria serve a signalling function via the production of pro-angiogenic reactive oxygen species (ROS) rather than act as a bioenergetic power source[12]. Instead, ECs are highly glycolytic, more so than other cell types, and generate up to 85% of their ATP via glycolysis[10] (Fig. 1a). In fact, their glycolytic rates are comparable to those in many cancer cells, known to have a high aerobic glycolytic metabolism[10]. ECs are glucose addicted, as glucose starvation or treatment with the glucose analogue 2-deoxy-D-glucose induces cytotoxicity[13]. Although quiescent ECs require substantial glycolysis for homeostatic maintenance, glycolysis is still increased (by twofold) when shifting to proliferation[10].

A recent report studied the role of glycolysis in vessel sprouting by focusing on 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase-3 (PFKFB3)[10]. Its kinase activity is 700-fold higher than its bisphosphatase activity and favours the production of fructose-2,6-bisphosphate (F2,6BP), an allosteric activator of phosphofructokinase-1 (PFK1), itself a rate-limiting enzyme of glycolysis (Fig. 1a). Inhibition of PFKFB3 reduces EC glycolytic flux levels, but by no more than 30–40%. Nonetheless, this is sufficient to impair vessel sprouting *in vitro* and induce vascular hypobranching *in vivo*[10]. Mechanistically, PFKFB3 deficiency in ECs reduces proliferation of stalk cells, in agreement with reports that PFKFB3-driven glycolysis is elevated in the G_1 cell cycle phase in preparation for the S phase. PFKFB3-driven glycolysis is also necessary for tip cell function (that is, migration) and competitiveness by providing energy for the remodelling of the cytoskeleton. Indeed, when ECs form lamellipodia to migrate, glycolytic enzymes are relocated to these protrusions, where they become associated with F-actin. This compartmentalization enhances local synthesis of ATP at the leading membrane ruffles in lamellipodia[10].

Treatment of mice with a PFKFB3 blocker reduces glycolysis only partially and transiently, yet impairs pathological angiogenesis, indicating that ECs are sensitive to small changes in glycolysis[14]. More complete and sustained inhibition of glycolysis by 2-deoxy-D-glucose impairs angiogenesis but is more toxic. Hence, partial and transient blockade of glycolysis may provide a new paradigm for anti-angiogenic therapy.

There are several hypotheses attempting to explain why ECs prefer glycolysis even though sufficient oxygen is present for oxidative metabolism ('aerobic glycolysis') and despite the fact that glucose oxidation is more efficient in yielding ATP (only 2 moles of ATP are generated via glycolysis versus up to 36 moles of ATP via glucose oxidation, per mole of glucose). First, by minimizing oxygen consumption, quiescent ECs reserve more oxygen for transfer to perivascular tissue cells. Second, ECs must vascularize avascular tissues, where low oxygen levels would preclude oxidative metabolism. Third, because of its high rate, glycolysis can generate more ATP than oxidative phosphorylation (OxPhos) as long as glucose availability is not limiting[15]. In fact, since interstitial oxygen levels drop faster than glucose levels over a distance away from a blood vessel, ECs

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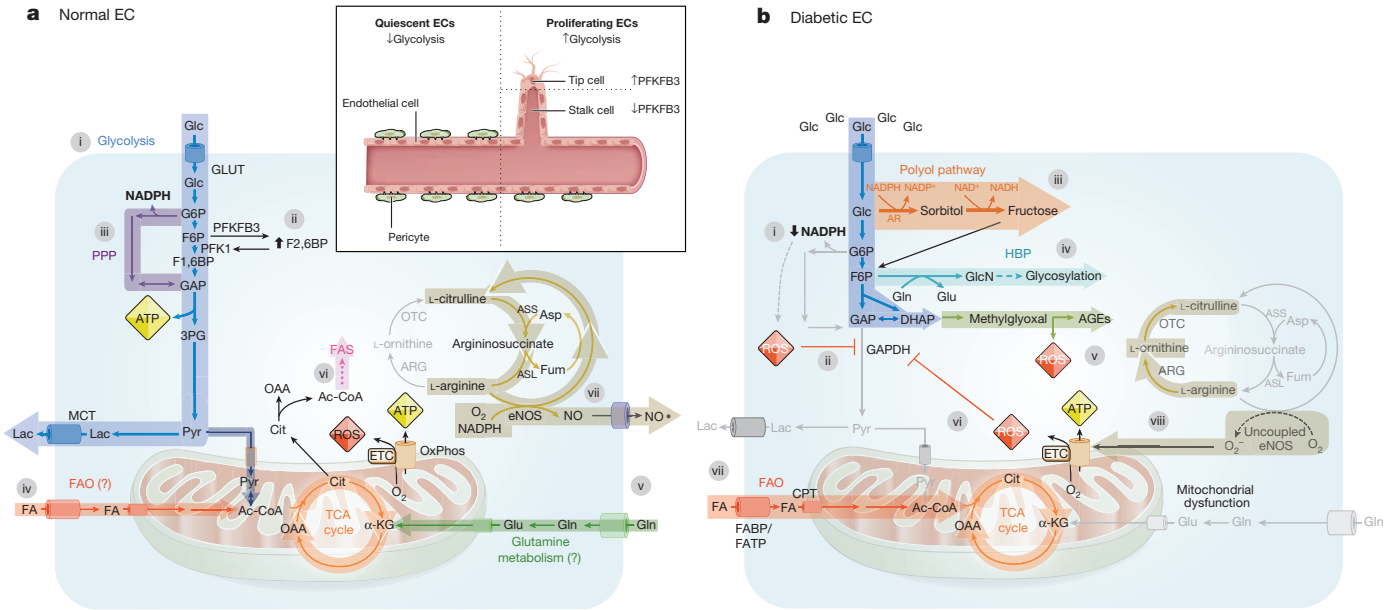

Figure 1 | Endothelial cell metabolism. For all figures: important metabolic pathways onto which cells rely are shown in colour; less critical or studied pathways are shown in grey. **a**, Scheme of the metabolic pathways in a normal EC. (i) ECs use glucose as primary source for energy production and are highly glycolytic (blue). Only a minor fraction of glucose is oxidized in the TCA cycle (orange). (ii) PFKFB3 is a regulator of glycolysis via conversion of F1,6BP to F2,6BP, an activator of PFK1. (iii) The PPP generates NADPH for redox homeostasis (purple). FAO generates acetyl-CoA (red) (iv), whereas glutamine metabolism generates α-KG (green) (v), which are metabolized in the TCA cycle (clockwise direction; orange). However, their importance versus glycolysis is unclear (denoted by question marks). (vi) ECs also synthesize fatty acids (FAS; pink). (vii) NOS converts arginine to nitric oxide (NO) and citrulline, which is recycled to regenerate arginine (tan). Inset: proliferating ECs have higher glycolysis than quiescent ECs; glycolysis is higher in tip than stalk cells. **b**, Metabolic changes in ECs in diabetes. (i) High glucose levels impair the PPP (tan), thereby lowering NADPH and elevating ROS levels. (ii) ROS reduces glycolysis by inactivating GADPH. (iii) This increases the polyol pathway flux (dark orange), leading to generation of ROS and toxic AGEs. (iv) The flux of the hexosamine biosynthesis pathway (cyan) is also increased, resulting in alteration of glycosylation. (v) GAP and DHAP are converted to methylglyoxal, leading to production of ROS and AGEs (olive). (vi) Excess mitochondrial ROS generation inhibits GAPDH, leading to accumulation of GAP and DHAP and reducing glycolysis. (vii) High glucose levels increase FAO at the expense of glucose oxidation. (viii) Increased arginase levels reduce the availability of arginine for the production of NO by NOS, which is uncoupled/less functional, and produces superoxide that induces mitochondrial dysfunction (tan). 3PG, 3-phosphoglycerate; α-KG, α-ketoglutarate; Ac-CoA, acetyl-coenzyme A; AGEs, advanced glycation end products; AR, aldose reductase; ARG, arginase; ASL, argininosuccinate lyase; Asp, aspartate; ASS, argininosuccinate synthase; Cit, citrate; CPT, carnitine palmitoyltransferase; DHAP, dihydroxyacetone phosphate; eNOS, endothelial nitric oxide synthase; ETC, electron transport chain; F1,6BP, fructose 1,6-bisphosphate; F2,6BP, fructose 2,6 bisphosphate; F6P, fructose 6-phosphate; FA, fatty acid; FABP, fatty acid binding protein; FAO, fatty acid oxidation; FAS, fatty acid synthesis; FATP, fatty acid transfer protein; Fum, fumarate; G6P, glucose 6-phosphate; GAP, glyceraldehyde 3-phosphate; Glc, glucose; GlcN, glucosamine; Gln, glutamine; Glu, glutamate; GLUT, glucose transporter; HBP, hexosamine biosynthesis pathway; Lac, lactate; MCT, monocarboxylate transporter; NADPH, nicotinamide adenine dinucleotide phosphate; NO, nitric oxide; OAA, oxaloacetate; OTC, ornithine transcarbamylase; OxPhos, oxidative phosphorylation; PFK1, phosphofructokinase-1; PFKFB3, 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase-3; PPP, pentose phosphate pathway; Pyr, pyruvate; ROS, reactive oxygen species; TCA, tricarboxylic acid.

can continue to rely on anaerobic glycolysis[16]. ECs are also resistant to hypoxia as long as glucose is available, but become sensitive to oxygen-deprivation when glucose is limiting[17]. Finally, glycolytic intermediates are used for anabolic generation of macromolecules and for redox control, which are necessary for EC proliferation, migration and survival[18]. Aside from their metabolic role, glycolytic metabolites (lactate) can stimulate angiogenesis also as signalling molecules (Box 1).

Role of additional metabolic pathways in ECs

The role of other metabolic pathways in angiogenesis *in vivo* remains less well defined (Fig. 1a). The pentose phosphate pathway (PPP), a side pathway of glycolysis, promotes cell proliferation by producing NADPH and ribose-5-phosphate, which are used for the synthesis of lipids, nucleotides and amino acids (histidine). The PPP also provides protection against oxidative stress by controlling redox homeostasis. Indeed, NADPH is used to convert the oxidized form of glutathione (GSSG) to its reduced form (GSH), a major antioxidant[19].

Another fuel used by proliferating cells is L-glutamine (referred to as glutamine), which has various metabolic fates, for example, oxidation of glutamine-derived α-ketoglutarate (α-KG) for energy production, glutaminolysis followed by pyruvate recycling into the tricarboxylic acid (TCA) cycle for continued TCA cycling, reductive carboxylation for lipid synthesis, or polyamine synthesis for mitogenesis (Fig. 1a). In line with findings that blockade of mitochondrial ATP production does not affect vessel sprouting[10], the importance of glutamine oxidation for the production of ATP in ECs is debated[10,20]. Nonetheless, glutamine metabolism is essential, as inhibition of glutaminase decreases proliferation and induces senescence of ECs[21,22]. ECs also use glutamine for the production of ornithine, a precursor of polyamines[23]. The role of glutaminolysis and reductive carboxylation in relation to vessel sprouting has not been studied to date.

Fatty acids can also serve as fuel for energy production, such as in oxidative cardiomyocytes. In metabolically stressed cancer cells, fatty acid oxidation (FAO) is vital for their survival by generating ATP and ensuring redox homeostasis (Fig. 1a)[24]. ECs also metabolize fatty acids for ATP production, but like for glutamine oxidation, the importance of FAO relative to glycolysis is debated[20,25]. Another role of FAO in ECs has not been described yet. Notably, proliferative cells also synthesize lipids, partly to form new membranes for cell division, to generate signalling molecules or to modulate signalling[26]. For instance, cholesterol promotes vessel sprouting by facilitating the formation of membrane lipid rafts in ECs, necessary for membrane localization and signalling of the pro-angiogenic VEGF receptor (VEGFR)-2 (ref. 27). Conversely, apolipoprotein-B lipoproteins, involved in cholesterol transport, impair angiogenesis

BOX 1

Signalling effects of metabolites and metabolic enzymes

Endothelial cells

Lactate, produced by cancer cells, promotes tumour angiogenesis via mechanisms independent of its role in glycolysis. By inhibiting the oxygen-sensing prolyl hydroxylase 2 (PHD2), lactate activates the hypoxia-inducible transcription factor HIF-1α in cancer cells and upregulates angiogenic signals[97]. Via the same mechanism, lactate triggers IκBα degradation, thereby stimulating an autocrine pro-angiogenic NF-κB/IL-8 pathway[98]. Furthermore, lactate activates the receptor tyrosine kinases AXL, TIE2 and VEGFR-2 in a ligand-independent manner[99]. In agreement, perturbing lactate influx in ECs by blocking the monocarboxylate transporter-1 (MCT1) inhibits tumour angiogenesis[21]. Interestingly, metabolic enzymes may also affect post-translational modification of targets. For instance, EC loss of fatty acid synthase (FASN) impedes angiogenesis by reducing palmitoylation of VEGFR-2 and eNOS, which impairs their plasma membrane targeting and activation[100].

Immune cells

Several metabolites have a signalling role in immune cells. For instance, high levels of the adenylate AMP in nutrient-poor conditions activate AMPK and control a metabolic checkpoint in T cells by acting as a brake on immune cell expansion when energy supplies are poor[55]. Another example is α-KG, which is a substrate for α-KG-dependent dioxygenases, such as PHDs, DNA hydroxylases and histone demethylases, whereas the TCA cycle intermediates fumarate and succinate inhibit PHDs via product-mediated inhibition. This stabilizes HIF-1α and induces a transcriptional program that induces a shift from oxidative to glycolytic metabolism in T_H17 cells and M1 macrophages[101]. Yet another example is kynurenine, a tryptophan metabolite generated by indoleamine 2,3-dioxygenase (IDO), expressed by dendritic, T_{reg} and endothelial cells, which suppresses CTLs and T_H1 cells while stimulating T_H2 polarization and T_{reg} cell recruitment[102]. Finally, glutathione released by dendritic cells is cleaved to cysteine, which promotes T_{eff} cell proliferation; T_{reg} cells inhibit this signalling[103]. Metabolic enzymes also engage in signalling in immune cells. For instance, GAPDH binds to the 3′ untranslated region of cytokine mRNAs (such as IFN-γ) and thereby dampens their expression[60]. Thus, when GAPDH is not supporting glycolysis for ATP production in T cells, it switches from its role as metabolic enzyme to an RNA-binding protein, thereby providing a metabolic checkpoint for T cells to couple effector status with glycolysis when sufficient nutrient is available[60].

by upregulating the expression of an anti-angiogenic VEGF-trap (that is, VEGFR-1) via unknown mechanisms[28]. Fatty acid synthesis is relevant for angiogenesis, as its inhibition impairs capillary formation[29].

The hexosamine biosynthetic pathway is a side branch of glycolysis, which produces *N*-acetylglucosamine, an intermediate in *N*-linked and *O*-linked glycosylation. As this pathway uses glutamine, glucose, acetyl-CoA, uridine and ATP, it functions as a nutrient sensor. ECs use this pathway to glycosylate key angiogenic molecules, such as VEGFR-2, Notch and others. Interfering with glycosylation has contextual effects, but can nonetheless impair angiogenesis[30].

Dysregulated metabolism of ECs in diabetes

EC dysfunction

The endothelium modulates vascular tone, structure and homeostasis. Maladaptive metabolic changes in ECs contribute to vascular complications in diabetes. Functional disruption of the endothelium in type 2 diabetes is an early event that promotes atherosclerosis and microangiopathy[2]. Notably, ECs are highly glycolytic at normal plasma glucose levels (5.5 mM), but high glucose levels (25 mM) render glucose metabolism of ECs maladaptive. In an EC line, high glucose increases FAO while decreasing glucose oxidation, a contextual finding requiring confirmation in primary ECs[31]. Various metabolic changes have been proposed to promote vascular complications in diabetics, largely by increasing oxidative stress due to generation of various types of ROS (such as superoxide (O_2^-•) and hydrogen peroxide (H_2O_2)) and reactive nitrogen species (including nitric oxide (NO)) (Fig. 1b). First, high glucose levels cause mitochondrial dysfunction and increase the production of ROS via protein kinase C-dependent activation of NADPH-dependent oxidases, a major source of ROS in diabetic ECs[32]. Second, ROS detoxification is impaired in ECs, as high glucose decreases the production of NADPH for redox control by inhibiting glucose-6-phosphate-dehydrogenase (G6PD), a rate-limiting PPP enzyme. Third, ROS indirectly inactivate the glycolytic enzyme GAPDH, which lowers glycolysis[33]. As a result of the reduced PPP and glycolytic flux, more glucose is shunted to the polyol pathway, which ECs use when exposed to high glucose in excess of what the glycolytic pathway can handle. Here, aldose reductase (AR) reduces glucose to sorbitol, which is further converted to fructose[34]. As AR converts NADPH to $NADP^+$, activation of this pathway depletes NADPH stores, which are necessary to maintain GSH levels for redox homeostasis, and thus promotes ROS accumulation. Sorbitol-derived fructose also generates the highly reactive 3-deoxyglucosone, which promotes the formation of advanced glycation end products (AGEs). Such glycated proteins and lipids contribute to vascular complications through formation of crosslinks between molecules in the basement membrane that alter the extracellular matrix. They also engage the receptor for AGEs (RAGE) on ECs, which induces vascular inflammation, leakage and further ROS production[35].

Fourth, ROS are generated when methylglyoxal is formed from the glycolytic metabolites dihydroxyacetone phosphate (DHAP) and glyceraldehyde-3-phosphate (GAP). The generation of methylglyoxal is enhanced in hyperglycaemic ECs because high glucose levels increase the formation of fructose (see above), which is metabolized to fructose-6-phosphate, and in turn to GAP and DHAP[36]. Production of methylglyoxal is also increased via inactivation of GAPDH by ROS, which again increases GAP and DHAP levels (Fig. 1b). Elevated plasma levels of methylglyoxal in diabetic patients exacerbate EC dysfunction, and induce the formation of AGEs, oxidative stress and EC apoptosis, in addition to impairing vascular reactivity, altogether contributing to vascular complications and hypertension in diabetics[37].

Fifth, high glucose levels impair endothelial nitric oxide synthase (eNOS) activity through increased *O*-linked glycosylation. They also uncouple eNOS, which instead of generating NO, now produces O_2^-•. By inhibiting cytochrome *c* oxidase in the mitochondrial electron transport chain, these reactive species promote EC dysfunction[38,39] (Fig. 1b). High glucose levels uncouple eNOS by increasing the levels of arginase, the enzyme that consumes L-arginine (referred to as arginine) necessary for the generation of NO (Fig. 1b).

Finally, ROS generated from high glucose exposure impairs angiogenesis via ligand-independent phosphorylation of VEGFR-2, leading to its reduced availability at the cell surface[40]. This was verified in an *ob/ob* mouse model of diabetes, and the effect was reversed by treatment of diabetic mice with the antioxidant *N*-acetyl-L-cysteine[40]. However, it should be cautioned that the precise contribution of oxidative stress to the development of vascular complications in diabetes remains debated. Also, despite all of this preclinical evidence, clinical antioxidant trials have not yielded overwhelmingly improved outcomes, highlighting the need for further study of these metabolic pathways[41]. Furthermore, studies on metabolic changes in diabetic ECs using metabolic tracing are warranted.

Ocular neovascularization

Diabetes impairs revascularization of ischaemic, inflamed and wounded tissues and impedes re-endothelialization of damaged vessels (such as after percutaneous coronary interventions), but also causes retinopathy characterized by overgrowth of leaky vessels inducing blindness[2]. Vessel

overgrowth in diabetic retinopathy is stimulated by ischaemia, an angiogenic stimulus. The retinal ischaemia results from vessel hypoperfusion caused by multiple vascular anomalies, including vessel disintegration due to vascular cell death, leakage due to impaired junctional integrity and bleeding due to rupture of microaneurysms[2]. Death of these vascular cells occurs as a result of excessive ROS production by an activated polyol pathway and mitochondrial dysfunction[42]. Increased glucose flux through the hexosamine biosynthetic pathway may also be involved, possibly via *O*-linked glycosylation of Akt[43]. From a clinical perspective, it is worth noting that glycaemic control reduces micro- but not macrovascular complications, suggesting a distinctive role of EC metabolism in different vascular beds[44]. Thus, it would be interesting to investigate whether the metabolism of micro- and macrovascular ECs differ.

Metabolism of healthy fibroblasts

Glycolysis and TCA cycle

Fibroblast metabolism has been studied only to a limited extent, but available evidence indicates that proliferating fibroblasts produce new biomass for replication, whereas quiescent fibroblasts generate biomass to replace oxidized lipids and degraded proteins, and to synthesize matrix proteins[45]. Glycolysis is higher in proliferating fibroblasts than quiescent fibroblasts, but only by twofold, indicating that quiescent fibroblasts have high glycolytic needs for baseline homeostasis[45] (Fig. 2a, b). Quiescent and cycling fibroblasts incorporate glucose carbons in the TCA cycle at comparable rates[45]. However, compared to proliferating fibroblasts in which glucose and glutamine metabolism in the TCA cycle is truncated at citrate, quiescent fibroblasts are able to use a fully operational TCA cycle. Quiescent and proliferating fibroblasts also differ in anaplerosis, a process that replenishes TCA cycle intermediates. Indeed, in quiescent fibroblasts, anaplerotic flux from pyruvate to oxaloacetate via pyruvate carboxylase ensures continuity of the TCA cycle, whereas proliferating fibroblasts primarily use glutamine for anaplerosis[45]. Further, reductive carboxylation of α-KG by mitochondrial isocitrate dehydrogenase (IDH)-2 is higher in quiescent fibroblasts, generating lipogenic acetyl-CoA for fatty acid synthesis[45] (Fig. 2a, b).

NADPH-producing pathways

Like cancer cells, proliferating fibroblasts rely on the PPP to generate ribose-5-phosphate and NADPH for biosynthesis, and incorporate glucose carbons via this pathway into nucleotides[45]. By contrast, quiescent fibroblasts use the PPP to generate NADPH for redox homeostasis, with glucose carbons flowing back to glycolysis as GAP and fructose-6-phosphate[45]. Quiescent fibroblasts also produce larger amounts of cytosolic NADPH by increasing the IDH-1 flux[45]. The role of the PPP in redox balance is essential for quiescence, as its inhibition induces apoptosis of quiescent but not of cycling fibroblasts[45].

Metabolism of cancer-associated fibroblasts

Cancer-associated fibroblasts (CAFs) promote the growth and metastasis of cancers. A bidirectional crosstalk between cancer cells and CAFs has been identified, whereby cancer cells highjack CAFs to produce high-energy nutrients for their own use[46]. In this crosstalk, production of ROS by cancer cells induces hypoxia-inducible factor 1α (HIF-1α) in neighbouring CAFs via inhibition of prolyl-hydroxylase domain (PHD) proteins. By inducing autophagy, HIF-1α promotes the degradation of caveolin-1, a negative regulator of NO production. Excess production of NO then inhibits cytochrome *c* oxidase and leads to dysfunctional mitochondria, which further elevates ROS levels in CAFs[46]. As CAFs remove dysfunctional mitochondria via mitophagy, they rely on enhanced glycolysis, leading to increased lactate production[46]. CAFs also generate amino acids and ketone bodies, which together with lactate are used by cancer cells in more oxygenated areas as high-energy nutrients of oxidative metabolism to generate ATP[47,48]. Via this metabolite crosstalk, CAFs thus feed nearby cancer cells. Proliferating CAFs also have increased rates of fatty acid synthesis, and blocking this process reduces their proliferation[49].

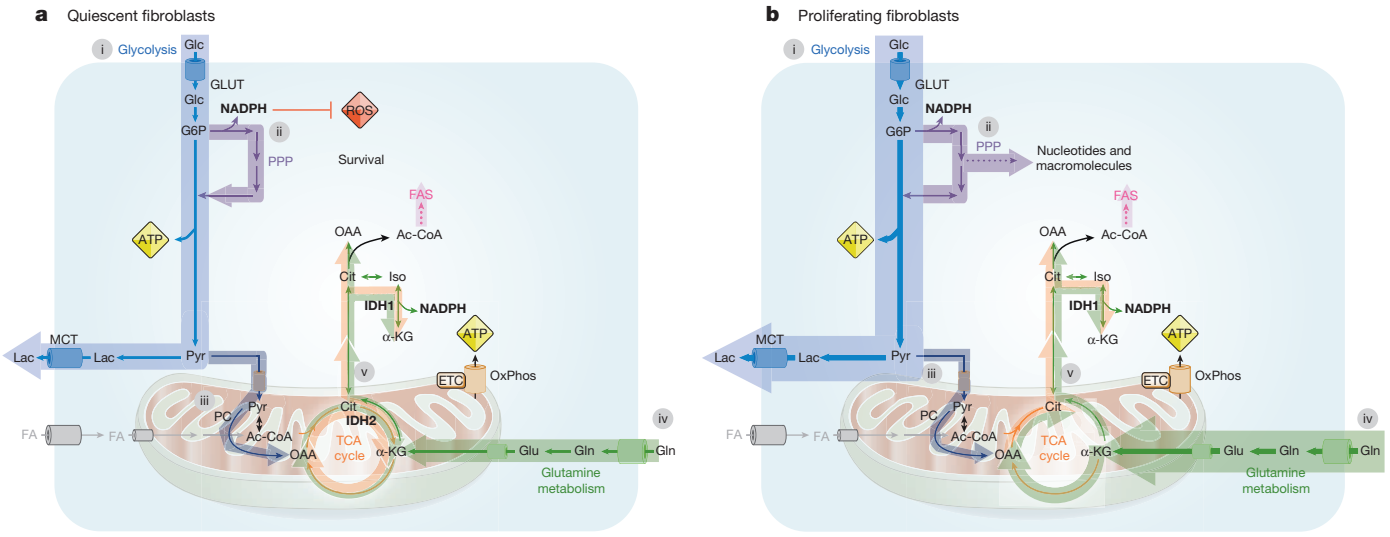

Figure 2 | Fibroblast metabolism. a, **b**, Scheme of the characteristic metabolic pathways in quiescent and proliferating fibroblasts. **a**, Quiescent fibroblasts: (i) have relatively high baseline glycolysis levels (blue). (ii) The PPP (purple) is important for redox homeostasis through enhanced production of NADPH (note that glucose carbons in the PPP flow back to glycolysis). (iii) Pyruvate carboxylase (dark blue) anaplerotically fills the TCA cycle with glucose carbons for glucose oxidation (orange), whereas (iv) glutamine is an anaplerotic carbon source for the TCA cycle (green; from glutamine to α-KG). (v) The TCA cycle proceeds in the forward (clockwise) direction. Quiescent fibroblasts have a high reductive carboxylation flux (green; from α-KG to citrate in anticlockwise reverse direction), generating acetyl-CoA for fatty acid synthesis (red) and NADPH for redox homeostasis by IDH1. Reductive carboxylation of α-KG, derived from glucose carbons, also occurs. **b**, Proliferating fibroblasts: (i) glycolysis (blue) is increased twofold in proliferating fibroblasts. (ii) The PPP is used to generate nucleotides and other macromolecules. (iii) Pyruvate carboxylase anaplerotically fills the TCA cycle with glucose carbons for glucose oxidation (dark blue), but (iv) glutamine is the major anaplerotic carbon source for the TCA cycle, consuming twice as much glutamine than quiescent cells (green; from glutamine to α-KG). (v) Proliferating fibroblasts have a truncated TCA cycle at citrate, preventing clockwise TCA cycling of glucose and glutamine carbons. However, compared to quiescent cells, they have a lower reductive carboxylation flux (green; from α-KG to citrate in the reverse direction anticlockwise), generating acetyl-CoA for FAS (red) and NADPH for redox homeostasis by IDH1. Iso, isocitrate; PC, pyruvate carboxylase; other abbreviations as in Fig. 1.

Immune cells

The innate immune system provides the first line of defence against infection by organisms bearing widely conserved pathogen motifs, whereas the adaptive immune system confers long-lasting antigen-specific protective immunity. The innate immune system is comprised of mast cells, macrophages, granulocytes (including basophils, eosinophils and neutrophils), dendritic cells and natural killer cells, whereas the adaptive immune system consists of B lymphocytes and T lymphocytes, including $CD4^+$ T helper (T_H) cells and $CD8^+$ cytotoxic T (CTL) cells. These immune cells contribute to tissue homeostasis in healthy conditions, but also influence tumour progression and metabolic disorders including type 2 diabetes[50,51]. We will discuss T cells and macrophages, for which metabolism has been best characterized.

T-cell metabolism

The T-cell response to immunogens occurs in three steps: priming, expansion and contraction. Naive T (T_n) cells are quiescent, but after engagement by an antigen-presenting cell, T_n cells become activated, or primed. Upon activation of the T-cell receptor (TCR), T_n cells undergo an initial growth phase (increase in cell size) during the first 24 h, followed by clonal expansion and differentiation over the next 24–72 h. During activation, they differentiate into a heterogeneous population of subsets of T effector (T_{eff}) cells. To divide every 6 to 8 h, and to produce effector molecules, T cells require increased amounts of energy and precursors for biomass production. Once the antigen is cleared, the T_{eff} cell population contracts and 90–95% of the activated T_{eff} cell pool dies, although a residual 5–10% of memory T cells (T_m in mice; T_{cm} (central memory) in humans) survives and provides immune memory; T_m cells can also differentiate from T_n cells[52]. By responding with greater speed and vigour to a repeat encounter with antigen, T_m cells protect the host without causing overt disease.

Metabolism during T-cell priming and expansion

T_n cells require minimal amounts of energy to prevent atrophy, survive and patrol the organism for antigen surveillance. Their ATP originates >90% from FAO and OxPhos, and to a lesser extent from glycolysis[53,54] (Fig. 3b). T_n cells also have low levels of glutamine anaplerosis[55].

Activation of T cells induces a coordinated increase of glucose and amino acid transporters, and upregulates glucose and glutamine catabolism (Fig. 3b). Indeed, primed T lymphocytes increase glycolysis by 20–50-fold, even in normoxia[56]. This increase stems partly from translocation of the glucose transporter GLUT1 to the plasma membrane and upregulation of glycolytic enzymes like hexokinase 2 and ADP-dependent glucokinase[56–59]. Glucose consumption by the PPP is also increased, although it is unclear for which purpose[54,58]. Aside from glycolysis, activated T cells also oxidize

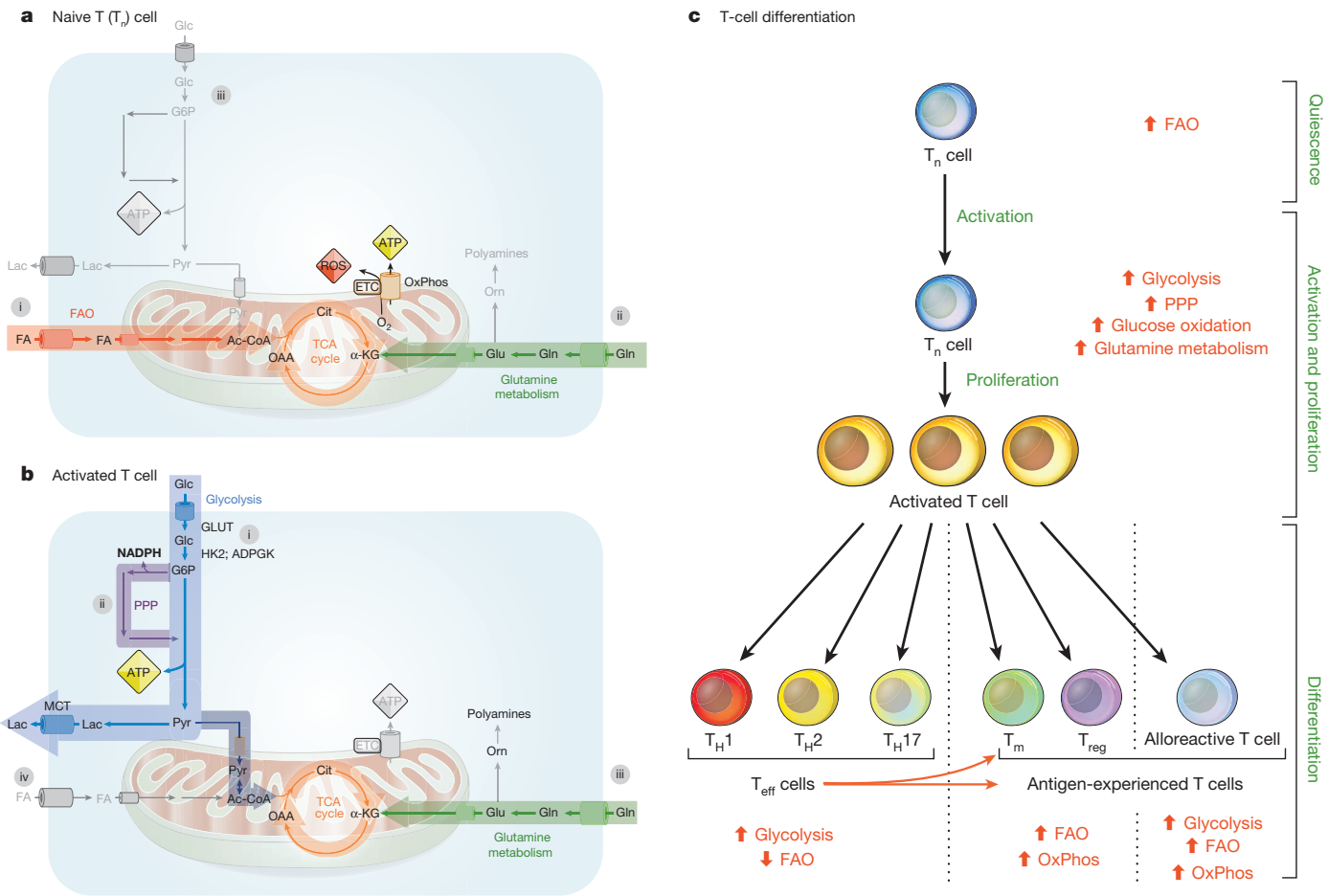

Figure 3 | T-cell metabolism. a, **b**, Metabolism of quiescent naive and activated T cells. **a**, (i) T_n cells rely primarily on FAO (red), (ii) baseline glutamine metabolism (green), (iii) but minimal glycolysis (grey). **b**, Upon activation, (i) they increase glucose uptake through GLUT1 and glycolysis by upregulation of hexokinase 2 (HK2) and ADP-dependent glucokinase (ADPGK) for ATP production. (ii) Increased PPP flux provides metabolites for redox homeostasis (NADPH) and proliferation (ribose-5-phosphate). (iii) Increased uptake of glutamine fuels the TCA cycle via anaplerosis (green), (iv) while FAO levels are reduced. The TCA proceeds in clockwise direction (orange). **c**, Metabolic changes upon activation (priming) and differentiation of T cells towards their different subtypes. For reasons of clarity, differentiation of T_{reg} and T_m cells from T_n cells is not shown. Unlike T_m or T_n cells, T_{reg} cells continuously proliferate at moderate levels. Orn, ornithine; other abbreviations as in Fig. 1.

glutamine-derived α-KG[60]. In addition, they metabolize glutamine via ornithine to mitogenic polyamines to promote their growth[58].

T-cell proliferation also relies on enhanced glucose and glutamine metabolism, and the importance of both pathways is exemplified by findings that deprivation of each nutrient blocks this process, even if other carbon sources are available[56,58]. Although OxPhos can fuel T-cell proliferation[60], increased glucose uptake and glycolysis, not OxPhos, are necessary to activate $CD4^+$ and $CD8^+$ T cells to full effector status upon TCR activation, an effect relying in part on signalling by the glycolytic enzyme GAPDH in $CD4^+$ T cells[60,61] (Box 1). By inducing ROS signalling, OxPhos can also affect $CD8^+$ T-cell expansion[62].

Proliferating cells often elevate fatty acid synthesis (see above). Although oxidation of glutamine-derived α-KG in the TCA cycle is only a minor source of lipogenic acetyl-CoA in normal conditions (when it is primarily derived from glucose metabolism), α-KG can be converted to citrate via reductive carboxylation in conditions of hypoxic stress or mitochondrial dysfunction[63,64]. T cells, which often operate in hypoxic wounds or inflamed tissues, preferentially use glutamine for biosynthesis of lipids by switching on reductive carboxylation[63]. In agreement, TCR activation increases fatty acid synthesis and induces a redistribution of arachidonate from phosphatidylcholine to phosphatidylethanolamine species[65]. Together, this coordinated metabolic switch promotes biosynthesis of proteins, nucleotides and phospholipids for rapid cell proliferation and effector status.

Metabolism during T-cell differentiation

Once T cells divide, they differentiate to different T-cell subsets, each switching on distinctive metabolic pathways. Activated $CD4^+$ T cells differentiate to subsets of T_{eff} cells: T_H1 cells mediate responses to intracellular pathogens, T_H2 cells control responses to helminths, and T_H17 cells are important in the defence against extracellular bacteria and fungi (Fig. 3c). T_{eff} cells upregulate glycolysis for anabolic purposes and suppress catabolic FAO for ATP production[66,67] (Fig. 3c). T_{eff} cells also require glutamine for late effector status[68]; however, the metabolic fate of glutamine oxidation remains unknown. Activated $CD8^+$ CTLs also upregulate glucose and glutamine catabolism, but unlike $CD4^+$ T cells, they do not increase OxPhos, making them more sensitive to glucose depletion[53,54].

Immune-suppressive regulatory T (T_{reg}) cells, another differentiated cell type, rely on FAO for ATP production[67,69]. Interestingly, these cells do not exhibit a sudden proliferation burst like T_{eff} cells, but continuously proliferate at moderate levels. Whether this might explain their dependence on FAO rather than glycolysis is unknown. T_{reg} cells also differ from T_H17 cells in their need for amino acid metabolism. Indeed, depletion of extracellular amino acids suppresses T_H17 cell differentiation, while favouring T_{reg} cell development and T-cell anergy (a long-term state of hyporesponsiveness in response to suboptimal stimulation)[70].

T_m cells adopt a metabolic profile comparable to that of T_n cells, but differ by increased mitochondrial mass and reserve respiratory capacity, which prepares them for rapid mitochondrial ATP production upon TCR re-engagement, and provides a bioenergetic advantage upon secondary antigen exposure[71]. Hence, specific metabolic programs in different immune cells are critical for multiple aspects and during specific phases of the immune response, with engagement of glycolysis and FAO during acute and more chronic antigen exposure, respectively.

The metabolic phenotype of activated T cells is influenced by the microenvironment. Indeed, during graft versus host disease (GVHD), allogenic T_{eff} cells increase not only glycolysis but also fatty acid uptake and FAO to support activation and proliferation *in vivo*[72]. Presumably, the prolonged presentation of antigens to T cells combined with other factors (for example, the type of antigen-presenting cell) during GVHD drives these cells to generate ATP as efficiently as possible by using oxidative metabolism as well (Fig. 3c). This is different from a condition whereby only limited amounts of antigens are presented for a finite period with minimal amounts of systemic inflammation, which T cells can handle by upregulating glycolysis alone. Signalling by metabolites and metabolic enzymes, and metabolite crosstalk, also regulate T-cell growth, differentiation and function (Box 2).

BOX 2

Metabolite crosstalks

Metabolite exchange between stromal and parenchymal cells is common and essential for the function of numerous tissues. For instance, extracellular lactate produced by ECs functions as a dynamic vasoactive signal for pericytes. Indeed, when cellular energy availability is low, lactate causes vasodilation to promote fuel supply, but when energy supplies are abundant, lactate acts as a vasoconstrictor[104]. This glycolytic metabolite, produced by cancer cells, also promotes angiogenesis, inhibits cytotoxic T-cell responses, and causes chronic inflammation through enhanced IL-17A secretion, which activates T_H17 cells[102]. As T_{eff} cells consume glucose intensely during early inflammation, glucose deprivation might promote T_{reg} cell differentiation to dampen inflammation[67]. Further, acidification of the tumour microenvironment by lactate increases arginase 1 levels in macrophages, which limits the anti-tumour immune response (see text)[105]. This effect can be abrogated by the glucose oxidation inhibitor dichloroacetate[105]. An analogous metabolic cooperation exists between cancer-activated adipocytes and cancer cells, where cancer cells reprogram the metabolism of adipocytes to become catabolic, so that they release fatty acids as fuel for cancer cells[106].

Macrophage metabolism

M1-like macrophages

Macrophages are terminally differentiated cells that do not need energy and biomass to proliferate but instead to sustain a high phagocytic and secretory activity. Macrophages are polarized to distinct phenotypes[73]. When activated by interferon (IFN)-γ alone or in concert with microbial stimuli (like LPS) or pro-inflammatory cytokines (like tumour necrosis factor (TNF)-α), they acquire an inflammatory phenotype, and are termed 'classically activated' M1 macrophages. These cells have a high microbiocidal activity, as well as enhanced production of pro-inflammatory cytokines and ROS. M1 macrophages increase glycolysis by inducing the expression of the pro-glycolytic PFKFB3 isoform[74] (Fig. 4a). Their dependence on glycolysis rather than mitochondrial ATP production, even in normoxia, offers M1 macrophages energetic advantages in hypoxic regions[75].

LPS has various effects on macrophage metabolism: first, it increases levels of the TCA intermediate succinate, which upregulates IL-1β expression via activation of HIF-1α (succinate inhibits PHDs that negatively regulate HIF-1α)[76]. Second, it increases succinylation of metabolic proteins, but the precise function of this modification remains unknown[76]. Third, LPS increases the expression of immunoresponsive gene 1 (IRG1), an enzyme producing itaconic acid[77] (Fig. 4a). Because itaconic acid inhibits isocitrate lyase, the key enzyme of the glyoxylate shunt in pathogens, this metabolite exerts antimicrobial activity during bacterial infections[77].

A key feature of M1 macrophages is their ability to produce high levels of mitochondrial ROS to kill phagocytosed bacteria. IRG1 enhances FAO and OxPhos to generate bactericidal mitochondrial ROS inside phagosomes containing ingested bacteria[78] (Fig. 4a). M1 macrophages also release ROS and reactive nitrogen species (including NO) in phagosomes for killing pathogens. ROS and NO for this purpose are generated by NADPH oxidase and inducible nitric oxide synthase (iNOS), respectively (Fig. 4a). Both enzymes require NADPH, which is produced in the PPP as well as by malic enzyme. The latter NADPH-producing mechanism is induced in response to LPS. In brief, LPS increases transport of mitochondrial citrate to the cytosol, where it is converted to oxaloacetate and then to pyruvate by malic enzyme, thereby generating NADPH[79]. To protect themselves against ROS leaking into the cytosol during such high oxidative burst, M1 macrophages also use NADPH from the PPP for the production of the antioxidant GSH (Fig. 4b).

To phagocytose, a large turnover of lipids and generation of membrane-rich filopodia and organelles are required. Macrophages therefore increase phospholipid synthesis and switch from cholesterol to phosphatidylcholine

production[80]. The importance of fatty acid synthesis is revealed by findings that its suppression prevents phagocytosis[80]. Citrate is also metabolized for the synthesis of phospholipids, a source of arachidonic acid for the production of pro-inflammatory prostaglandin[79].

M2-like macrophages

'Alternatively activated' M2 macrophages are associated with tissue repair, angiogenesis and pro-allergic and anti-parasitic T_H2 immunity, as well as with anti-inflammatory cytokine production and reduced expression of major histocompatibility complex class II and antigen presentation[73]. They have low glycolysis rates and no detectable PFKFB3 levels, expressing the weak glycolytic activator PFKFB1 instead, but have high rates of FAO and OxPhos[81] (Fig. 4b). The M2-macrophage-inducing signal IL-4 stimulates mitochondrial biogenesis by upregulating PGC-1β, thereby enhancing a metabolic switch to FAO[82]. Also, by stimulating angiogenesis and tissue oxygenation, M2 macrophages prepare for oxidative metabolism. Of note, a high lipid content, as occurs upon impairment of cholesterol efflux, promotes the angiogenic M2 phenotype[83], further establishing the link between lipid metabolism and M2 polarization. Thus, the M2 macrophage secretory program is energetically demanding, both in terms of intensity and duration, and oxidative metabolism is best suited to meet the bioenergetic demands for long-term macrophage activation.

M2 macrophages decrease the PPP flux and GSH levels via upregulation of carbohydrate kinase-like protein (CARKL, also known as SHPK). This kinase lowers the oxidative PPP flux by catalysing the formation of sedoheptulose-7-phosphate, a PPP inhibitor[84] (Fig. 4b). It has been proposed that M2 macrophages decrease the PPP flux, because the activity of key inflammatory regulators might be sensitive to the redox state[84]. Overall, it is becoming increasingly evident that intracellular metabolic changes are necessary to determine the activation status and function of macrophages, but also that macrophages can alter metabolic functions of other cells in the environment (Box 2)[85].

Macrophage metabolism in cancer

Tumours harbour distinct types of tumour-associated macrophages (TAMs), that is, M1 TAMs suppressing and M2 TAMs promoting tumour growth[73]. Little is known about specific metabolic adaptations of TAMs. They differ, however, in their use of arginine. Indeed, M1 TAMs express iNOS, which converts arginine to NO, which is cytotoxic for tumour cells[86] (Fig. 4a). By contrast, M2 TAMs express arginase 1, which metabolizes arginine to ornithine (a precursor of polyamines), which promotes M2 polarization and can stimulate nearby cancer cells[86] (Fig. 4b). By upregulating arginase 1, M2 TAMs impair the anti-tumour activity of T cells as this depletes the arginine pool for NO and protein synthesis, which impairs TCR function[87]. However, the role of arginases in polyamine production in macrophages requires further study, as polyamines can be generated in their absence.

Metabolism determines cell differentiation

Cancer cell metabolism studies have focused on understanding how malignant cells adopt their metabolism to sustain increased proliferation rates. Emerging evidence from studying non-transformed endothelial and immune cells now shows that metabolism not only supports cell proliferation, but also seems to be a determinant of cell differentiation. Nonetheless, unravelling whether the metabolic signature of differentiated cell types is simply a consequence of lineage/phenotype-specific signalling or rather is instructive for regulating cell plasticity and/or function remains a question for the field.

Endothelial cells

Vascular sprouting relies on collective EC migration, whereby the leading endothelial tip cell at the vascular front guides the sprout and trailing proliferating stalk cells elongate the sprout[1]. As the pro-tip cell signal VEGF elevates PFKFB3 expression, PFKFB3 levels are higher in tip than in stalk cells[10] (Fig. 1a inset). PFKFB3 overexpression is capable of overruling Notch signalling, thereby empowering stalk cells to become tip cells[10]. This is

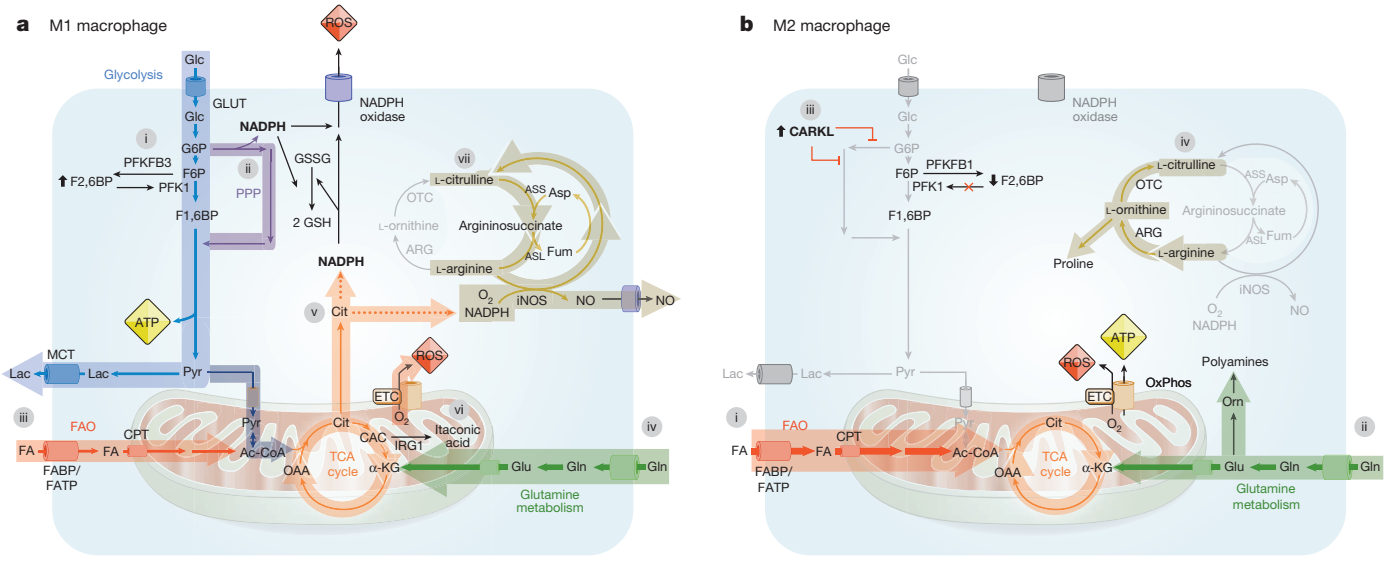

Figure 4 | Macrophage metabolism. **a**, M1 macrophages: (i) are characterized by high glycolysis rates (blue) via activation of PFKFB3, and (ii) by increased PPP (purple), generating NADPH, used for redox homeostasis and production of ROS by NADPH oxidase; mitochondria also produce ROS. (iii) FAO (red) is present basally, (iv) whereas glutamine (green) anaplerotically fuels the TCA cycle. (v) Mitochondrial export of citrate is important for NO and ROS generation (orange). (vi) The TCA cycle intermediate *cis*-aconitate (CAC) is converted to itaconic acid by immunoregulatory gene 1 (termed *cis*-aconitate decarboxylase; IRG1), which is important for mitochondrial ROS production from FAO for bactericidal clearance. (vii) Arginine metabolism by iNOS generates NO and L-citrulline, used to regenerate arginine via the activity of argininosuccinate synthetase and argininosuccinate lyase (tan). **b**, M2 macrophages: (i) rely on FAO (red), whereas glycolysis (grey) is low owing to expression of PFKFB1. (ii) Glutamine is used for the synthesis of polyamines or enters the TCA cycle for oxidative metabolism (green). Acetyl-CoA generated in FAO and α-KG derived from glutamine are metabolized in the TCA cycle (forward direction; orange). (iii) The PPP (grey) is reduced by carbohydrate kinase-like protein (CARKL). (iv) Arginase metabolizes arginine to produce ornithine, which is converted to polyamines or L-citrulline (tan). GSSG, oxidized glutathione; GSH, reduced glutathione; iNOS, inducible nitric oxide synthase; PFKFB1, 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase-1; other abbreviations as in Fig. 1.

remarkable, as Notch is the most potent pro-stalk signal known to date. Thus, PFKFB3-driven glycolysis regulates the plasticity of EC phenotypes, and not only genetic but also metabolic signals regulate vessel sprouting.

Immune cells

Metabolism also controls T-cell lineage choices. In accordance with the importance of glycolysis for T_{eff} cells, activation of glycolysis by transgenic expression of GLUT1 or glycolytic genes augments T-cell activation, results in the accumulation of readily activated T_m-like cells with signs of autoimmunity in aged mice[56], and drives $CD8^+$ T cells towards a terminally differentiated, more short-lived state[88]. Conversely, blocking glycolysis via pharmacological or transgenic means impairs T_H17 cell proliferation and differentiation while promoting T_{reg} cells, thereby protecting mice from autoimmune inflammation[58,66]. Moreover, inhibiting glucose metabolism preserves the formation of long-lived $CD8^+$ T_m cells *in vivo*[88], while rendering T cells anergic[54].

Amino acid and fatty acid metabolism are also important, as inhibition of glutaminase impairs T_{eff}-cell proliferation[58], whereas FAO blockade inhibits T_{reg} differentiation[67] and the survival of alloreactive T cells[72]. Conversely, the short-chain fatty acid butyrate, produced by commensal microorganisms, promotes T_{reg}-cell development in the colon, thereby protecting mice against colitis[89–91]. Also, by mediating leucine uptake, the amino acid transporter SLC7A5 induces $CD4^+$ and $CD8^+$ T-cell differentiation via activation of mammalian target of rapamycin complex 1 (mTORC1), glycolysis and glutamine metabolism, thereby functioning as a metabolic checkpoint[92]. Macrophage differentiation is also determined by metabolism. Indeed, pretreatment of macrophage precursors with respiration or FAO blockers abrogates M2 activation, without affecting the M1 phenotype[81]. Aside from a role in metabolism, metabolites and metabolic enzymes modulate the plasticity of immune cells by affecting gene transcription, signalling pathways or epigenetic programs (Box 2).

Metabolic commonalities and differences

This overview highlights that ECs, fibroblasts and immune cells both share and differ in their use of metabolic pathways, although metabolism is incompletely characterized in some of these cell types. For instance, proliferating endothelial stalk cells, fibroblasts, T_H1 cells, T_H2 cells and T_H17 cells all rely on increased glycolysis, glutamine metabolism and fatty acid synthesis to generate the needed amount of ATP and new biomass for cell division. Endothelial tip cells, T_{eff} cells and activated M1 macrophages also rely on glycolysis, but use it instead to achieve full effector status. By contrast, T_m cells, T_{reg} cells, alloreactive T cells and M2 macrophages rely primarily on FAO to generate ATP more efficiently. Oxidative metabolism seems to be best suited to meet the bioenergetic demands for the long-term performance of these cells. Reductive carboxylation of α-KG for fatty acid synthesis has only been identified to date in M1 macrophages and fibroblasts, but is probably also relevant for other non-transformed cell types.

The PPP is of vital importance for many cells, but is used for distinctive purposes in different stromal and immune cell types. For instance, the PPP is essential for biosynthesis of macromolecules in proliferating fibroblasts and probably also in other dividing cells (ECs, T cells), although evidence is lacking so far. The PPP and other NADPH-generating pathways (including FAO) are used by M1 macrophages for the production of ROS to kill pathogens. To buffer this oxidative burst, they must also use the PPP for redox homeostasis. Quiescent fibroblasts are unusually sensitive to redox balance offered by the PPP. ROS have a signalling role in ECs and T cells, unlike their lethal bactericidal effects in M1 macrophages.

Quiescent ECs and fibroblasts differ from T_n cells in their needs for glycolysis during homeostatic maintenance. This may relate to the fact that quiescent T_n cells are largely inactive, awaiting activation by foreign antigen, whereas quiescent ECs and fibroblasts must continuously maintain the vascular barrier, transport molecules, synthesize matrix, replenish degraded macromolecules and perform homeostatic activities. Unlike other stromal and immune cells, macrophages do not use metabolism for division, but to resynthesize degraded biomass and achieve full effector status. Overall, stromal and immune cells have adopted their metabolism to optimally fulfil their distinctive role as support cells in particular conditions, in which each of these stromal and immune cells must fulfil specialized functions.

Outstanding questions and perspectives

We suspect that the studies discussed here merely scratch the surface of the metabolic control of stromal and immune cells. Multiple questions require an answer in the future. A priority for EC and fibroblast metabolism research is to establish a road map of additional metabolic pathways that have not been studied to date. It will be equally fruitful to study how stromal and immune cell types metabolically communicate with each other and other (cancer) cells, and whether pathways or signalling properties of metabolic enzymes, characterized in cancer cells, also exist in stromal and immune cells. Analysing the metabolism of these cell types *in vivo*, in preclinical animal models and ultimately in patients is an important yet challenging translational priority for the future. Furthermore, little is known about the metabolism of various stromal cell types (for example, arterial, venous, lymphatic or tumour ECs; pericytes and smooth muscle cells; bone marrow mesenchymal cells, hepatic stellate cells) and other immune cells (for example, neutrophils, basophils, monocytes, dendritic cells) or of their progenitors, and whether metabolism determines their phenotypic identity. For instance, do tip and stalk ECs have distinct metabolic signatures (besides a difference in glycolysis), and do pro-tip or pro-stalk cell signals determine specific metabolic pathways?

Another question is whether stromal and immune cell metabolism differs from cancer cell metabolism. It has been postulated that the unique properties of cancer cell metabolism would offer opportunities to selectively target cancer cells. Although this seems plausible for tumours in which metabolism is changed by genetic alteration or overexpression of metabolic targets, it is unknown whether blocking metabolic changes more commonly seen in other tumours will target cancer cells selectively. Although metabolic similarities in cancer and stromal/immune cells may enhance anti-cancer therapies targeting a common metabolic pathway, possible consequences on healthy stromal cells, which also rely on these pathways, will need to be evaluated.

Finally, what are the translational consequences of these insights into stromal and immune cell metabolism for malignant, metabolic or inflammatory disease? Will it be possible to target particular stromal or immune cells, and target only activated or proliferating cells without affecting quiescent cells? Reports that blocking glycolysis protects mice from autoimmune inflammation by impairing T_H17 cell proliferation and differentiation, while favouring T_{reg} cell differentiation, suggest that this might be possible[58,66]. Another example is the transient and partial reduction of glycolysis by PFKFB3 blockade, which is sufficient to reduce pathological angiogenesis, without affecting pre-established vessels or evoking overt toxicity or metabolic compensation[14].

In preclinical models, lowering glycolysis improves psoriasis[93], protects against autoimmune inflammation[58,66], reduces ocular neovascularization (a common complication in diabetics)[14] and decreases tumour growth by inhibiting the growth of cancer cells and probably of ECs, fibroblasts and T cells[94]. In addition, glycolytic blockade enhances the anti-angiogenic effect of VEGF inhibitors, indicating that targeting metabolism can amplify available treatments[14]. The preclinical finding that inhibition of AR improves vascular complications in diabetes[95] has been translated to the clinical situation, and treatment of human diabetics with the AR inhibitor epalrestat prevents further progression of retinopathy[96]. These examples illustrate the therapeutic potential of targeting metabolism in stromal and immune cells, and should provide an incentive to further characterize metabolism in these cells.

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